Certificate of Analysis

FLAG® Peptide

Catalog Numbers	Size
IB 13070	4mg
8230575	25mg

Description:

- Synthetic 1013 Dalton octapeptide: *N-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys-C;*

for competitive displacement of amino-terminal, Met-amino-terminal or carboxy-terminal FLAG fusion proteins from the Anti-FLAG M2 Monoclonal Antibody in solution or bound to agarose on the Anti-FLAG M2 Affinity Gel.

Storage and Handling:

- Store lyophillized powder desiccated at +4°C until ready for use. Dissolve in 0.8 ml TBS (10mM Tris-HCl and 150 mM NaCl at a final pH of 7.4) immediately before use. Aliquot and store the unused peptide at -20°C. Do not repeatedly freeze-thaw.

Quality Assurance

Purity: >95% by Reverse Phase HPLC

Amino acid composition: As expected

Mass: mg FLAG peptide determined by Absorption at 274.8nm of Tyr (ε =1.405 x 10^{3}) of FLAG octapeptide.

Competitive Displacement:
25 Column Equivalents of FLAG peptide are sufficient to elute >90% of FLAG-BAP fusion protein from a 1ml Anti-FLAG M2 Affinity Gel loaded to capacity in 5x2 ml fractions each containing 5 Column Equivalents.

1 Column Equivalent is defined as the number of nmol of FLAG peptide required to saturate both antigen binding sites of all Anti-FLAG M2 Monoclonal Antibody bound to the Anti-FLAG M2 Affinity Gel.

To calculate column equivalents of FLAG peptide use the following equation:

1 Column Equivalent =

$$\frac{\text{mg MAb}}{\text{MW MAb}} \cdot \frac{1\text{g MAb}}{10^{3}\text{mg MAb}} \cdot \frac{2\text{mol FLAG peptide}}{\text{mol MAb}} \cdot \frac{10^{9}\text{-nmol FLAG peptide}}{\text{mol FLAG peptide}}$$

=mg MAb x 12.5 nmol FLAG peptide

CERTIFIED

Item: FLAG Peptide

Cat#: IB13070

Lot#: 8C0322

Where:
mg MAb =mg Anti-FLAG M2 Monoclonal Antibody attached to the Affintiy Gel.

MW MAb = molecular weight of Anti-FLAG M2 MAb = 160,000 g/mol

For Additional Information or to Place an Order

Please call Kodak Scientific Imaging Systems at (800) 225-5352 or (716) 722-5813 if calling from outside of the U.S.

**BAP: Bacterial Alkaline Phosphatase*
FLAG and Anti-FLAG are Trademarks of Immunex Corporation.

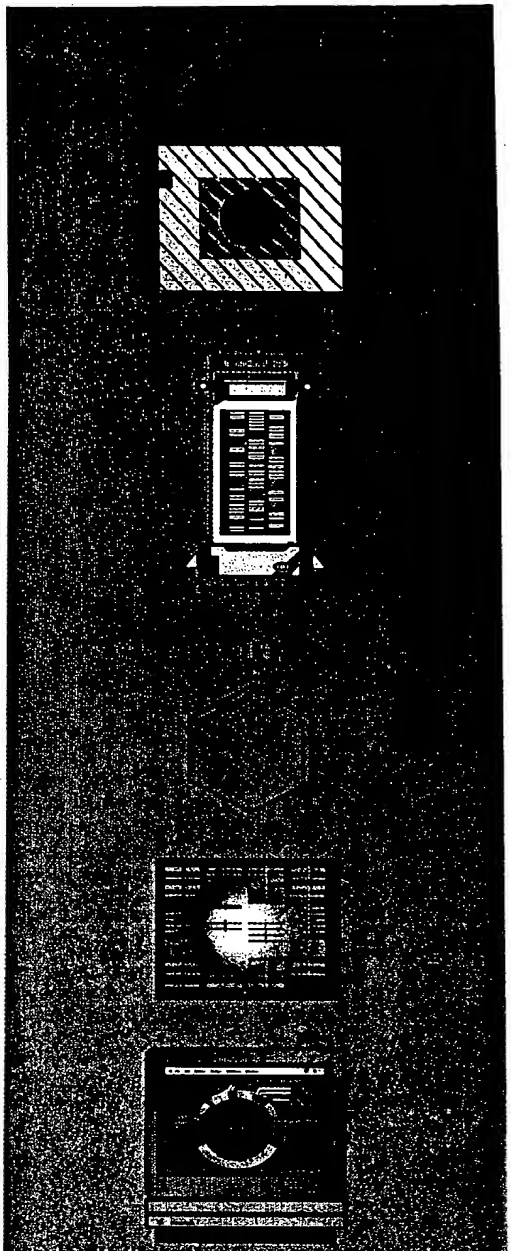
FOR RESEARCH USE ONLY

4/97

EASTMAN KODAK COMPANY
Scientific Imaging Systems
4 Science Park, New Haven, CT 06511

Applicants: Nika Adham , et al.
U.S. Serial No.: 09/116,676
Filing Date: July 16, 1998

Exhibit A



Baxter

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Craig Dunn
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Industrial

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100 Raritan Center Parkway
Edison, NJ 08818

908.225.4700
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800.888.6776 Cust. Serv.

BIOLOGICAL RESEARCH AND IMAGING PRODUCTS

CATALOG 1994/95

Kodak

Scientific Imaging Systems

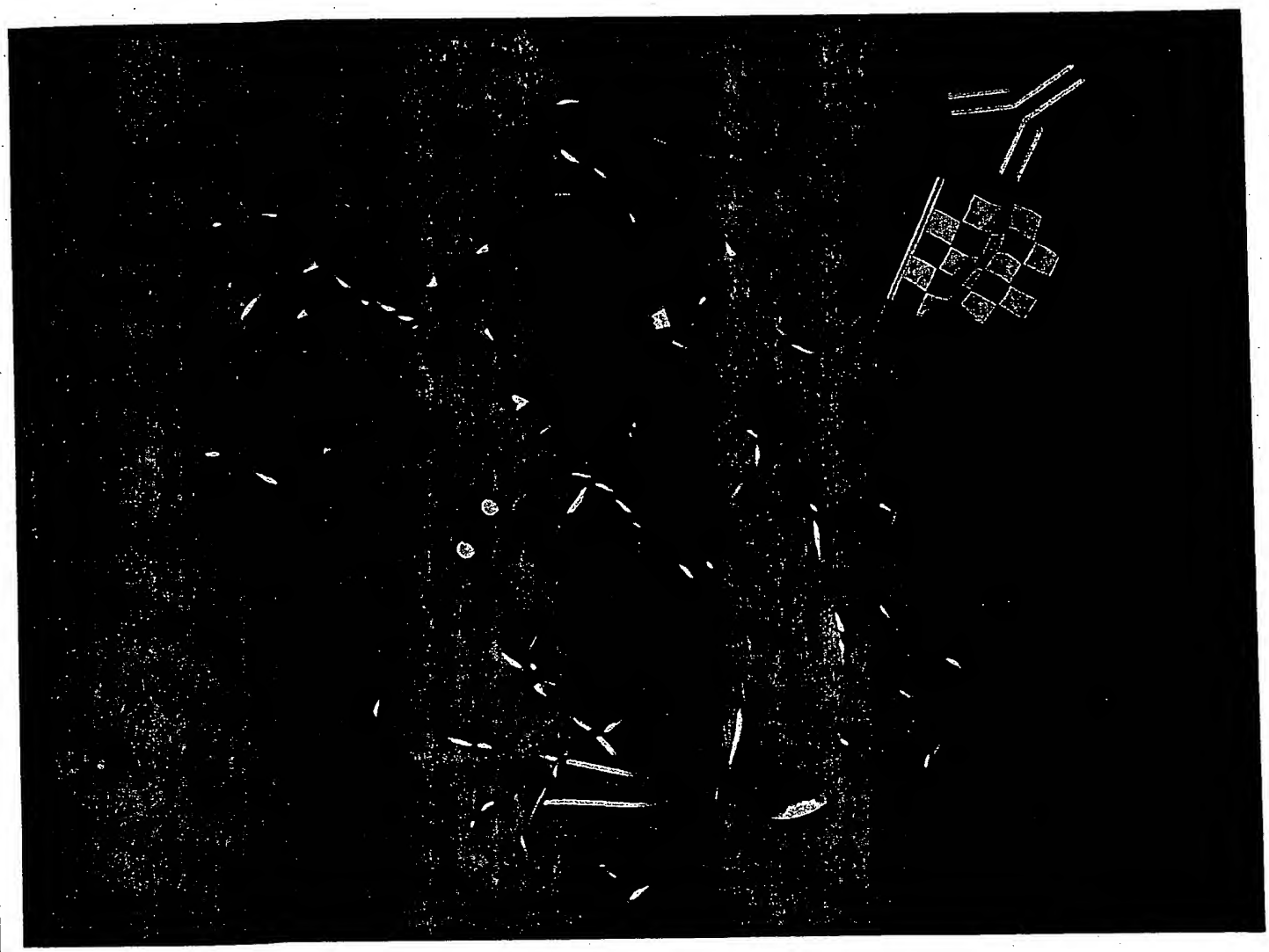
EASTMAN KODAK COMPANY
25 Science Park, New Haven, CT 06511

Table of Contents

CHAPTER 1

PROTEIN EXPRESSION, DETECTION AND PURIFICATION SYSTEMS

IBI FLAG® SYSTEM



Ribbon View Of Bacterial Alkaline Phosphatase[1]

The FLAG® Epitope was placed at the amino-terminus of Bacterial Alkaline Phosphatase (BAP) by substituting it for the first four amino acids of the native sequence. In this ribbon stereo view of one subunit of the BAP homodimer the location of the amino-terminal FLAG substitution is marked by the checkered FLAG at the upper right of the view. FLAG•BAP is the model fusion protein used for new product development. It serves as a positive control in all FLAG Expression Kits.

Coordinates for BAP were kindly provided by Hal Wcykoff, Department of Molecular Biophysics and Biochemistry, Yale University and the ribbon drawing was generated by John Spurlino at the Molecular Structure Center at Sterling-Winthrop Company.

(1) Eunice E. Kim and Harold W. Wyckoff, Reaction Mechanisms of Alkaline Phosphatase Based on Crystal Structures: Two Metal Ion Catalysis, J. Mol. Bio 218: 449 - 464 (1991).

IBI FLAG® SYSTEM

The IBI FLAG system is based on the fusion of an eight amino acid FLAG marker peptide to a cloned protein. (FIGURE 1). Fusion occurs by cloning the 24 base pair FLAG coding sequence adjacent to the appropriate protein coding sequence for expression by an animal, yeast, insect or *E. coli* FLAG expression vector. The FLAG peptide is recognized by the Anti•FLAG M1 and M2 Monoclonal Antibodies. An Anti•FLAG monoclonal antibody can be used for the immunological detection of the FLAG fusion protein in a wide variety of applications. The FLAG fusion protein is purified using an Anti•FLAG M1 or M2 Affinity Gel. Enterokinase is used to proteolytically remove the FLAG peptide from the FLAG fusion protein.

Benefits

The FLAG marker peptide has several features which make it useful for the affinity purification and immunological detection of recombinant FLAG fusion proteins:

• **Efficient:** Development of a specialized scheme or functional assay is not required to purify the protein. Antibodies do not have to be raised against the protein.

• **Versatile:** Amino-terminal or carboxy-terminal FLAG fusion proteins can be expressed in *E. coli*, yeast, insect or animal cells.

• **Minimal effect on protein function:** The small octapeptide has minimal effect on the conformation of the native protein.

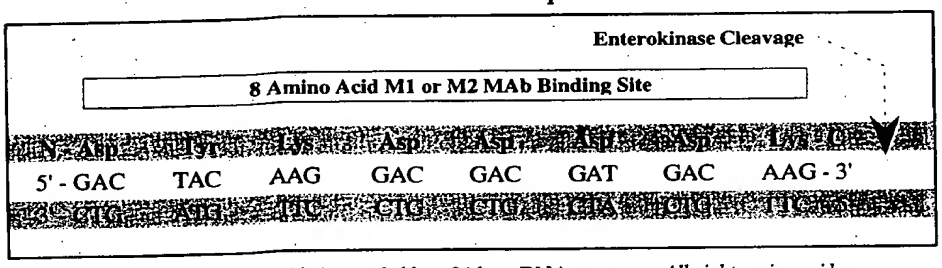
• **Ease of detection:** The eight amino acid sequence has a high surface probability. A surface location maximizes its accessibility to the Anti•FLAG M1 and M2 Monoclonal Antibodies.

• **Mild purification:** Rapid affinity purification of FLAG fusion proteins with the Anti•FLAG M1 or M2 Affinity Gel employs mild conditions for recovery of a biologically active protein.

• **Multiple applications:** Useful for further study of protein•protein interactions, protein•DNA interactions, protein surveillance and ultrastructure.

• **Ease of removal:** Contains the rare, five amino acid recognition sequence for enterokinase. This enables recovery of an intact protein following its proteolytic removal.

Figure 1:
FLAG Marker Peptide



The eight amino acid FLAG peptide is encoded by a 24 base DNA sequence. All eight amino acids are required for binding of the Anti•FLAG M1 or M2 Monoclonal Antibody (MAb). The enterokinase recognition site corresponds to the carboxy-terminal five amino acid sequence: Asp•Asp•Asp•Asp•Lys.

Applications:

The FLAG System has been employed for the affinity purification and immunological detection of FLAG fusion proteins in *E. coli*, yeast, insect and animal cells. (TABLE 1).

TABLE 1:
Applications of FLAG Technology

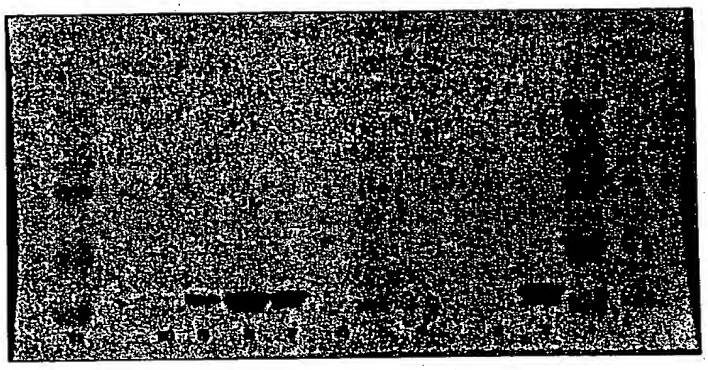
	E. coli	Yeast	Insect	Animal
Western Blot	1,2,4,11, 13,14,17,18	1,2		9, 12
Dot Blot	1,2			
Slot Blot	1,2			
Immunoprecipitation	1,			8,9,10,15,16,19
ELISA	1,2,20	1,2		
Light Microscopy				2,15
Fluorescence Microscopy			22	4,5,6,15,16
Electron Microscopy				21
FACS				4,6,23
Biosensor	24			
Gel Retardation				6-9
Affinity Purification	1,2,3,4,11,13,24	1,2		3,4,5

Numbered journal references are listed on page 1-14 and 1-15.

FLAG® SYSTEM

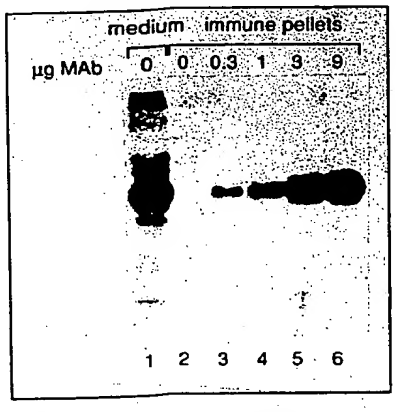
The Anti•FLAG M1 and M2 Monoclonal Antibodies have been used in several immunological detection procedures. These include immuno-blotting (FIGURE 2); immunoprecipitation and immuno-coprecipitation (FIGURE 3); ELISA (enzyme linked immunosorbent assay) (FIGURE 4); light microscopy (FIGURE 5);

FIGURE 2:
Western Blot



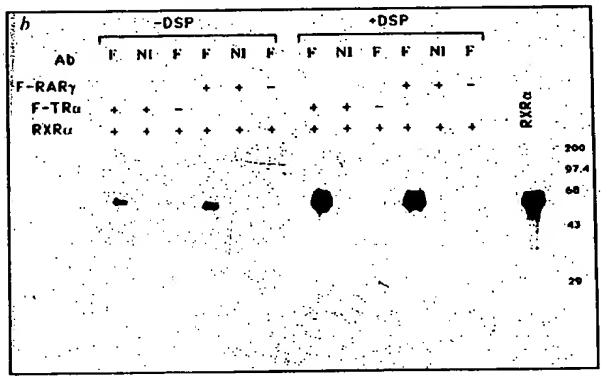
C-terminal Single Chain Antibody with Anti•FLAG M2 Monoclonal Antibody[18]

FIGURE 3a:
Immunoprecipitation



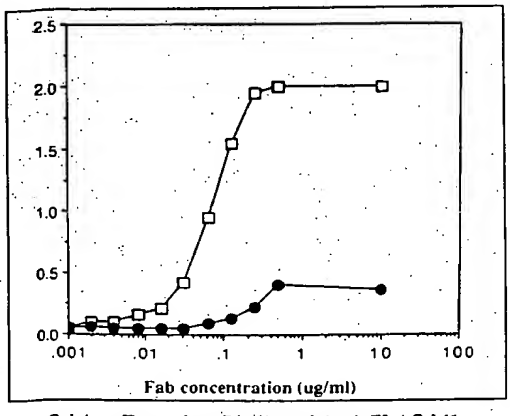
Titration of Met•FLAG Apolipoprotein B with Anti•FLAG M2 Monoclonal Antibody[19]

FIGURE 3b:
Immuno-coprecipitation



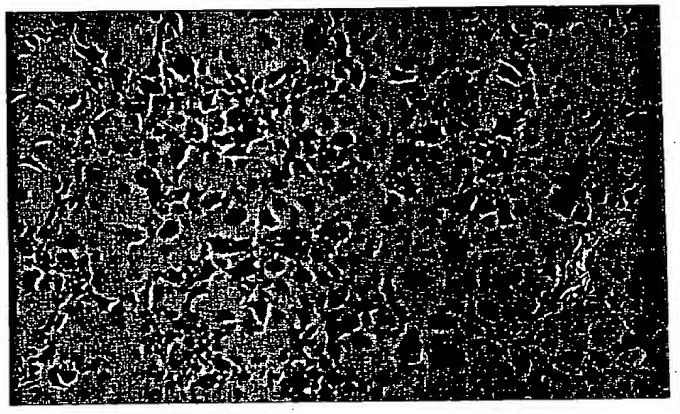
Interaction of RXRα and Thyroid Hormone Receptor α using the Anti•FLAG M2 Monoclonal Antibody[7-9]

FIGURE 4:
ELISA



Calcium Dependent Binding of Anti•FLAG M1 Monoclonal Antibody to FLAG•CSF[1-2,20]

FIGURE 5:
Light Microscopy



Detection of Signal Peptidase in COS Cells with the Anti•FLAG M2 Monoclonal Antibody[21]

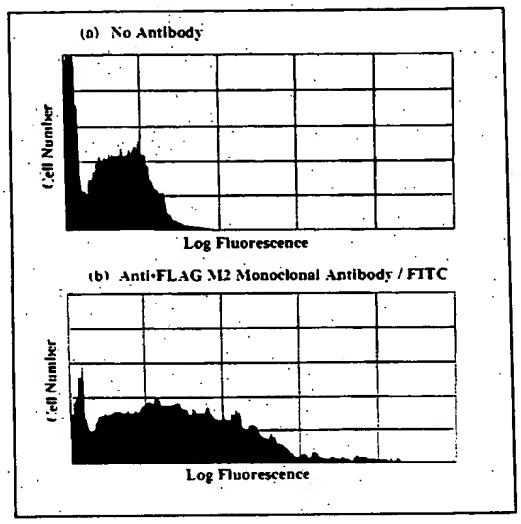
FLAG® SYSTEM

Fluorescence microscopy (FIGURE 6); FACS (fluorescence activated cell sorting) (FIGURE 7) and gel retardation / mobility shift (FIGURE 8), electron microscopy and biosensor. Anti•FLAG M1 and M2 Affinity Gels have been employed for the purification of several FLAG fusion proteins. (FIGURE 9).

FIGURE 6:
Fluorescence Microscopy

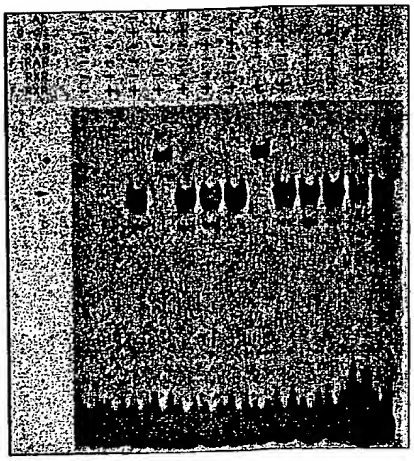
Detection of CFTR Channel Protein with the Anti•FLAG M2 Monoclonal Antibody [22]

FIGURE 7:
FACS



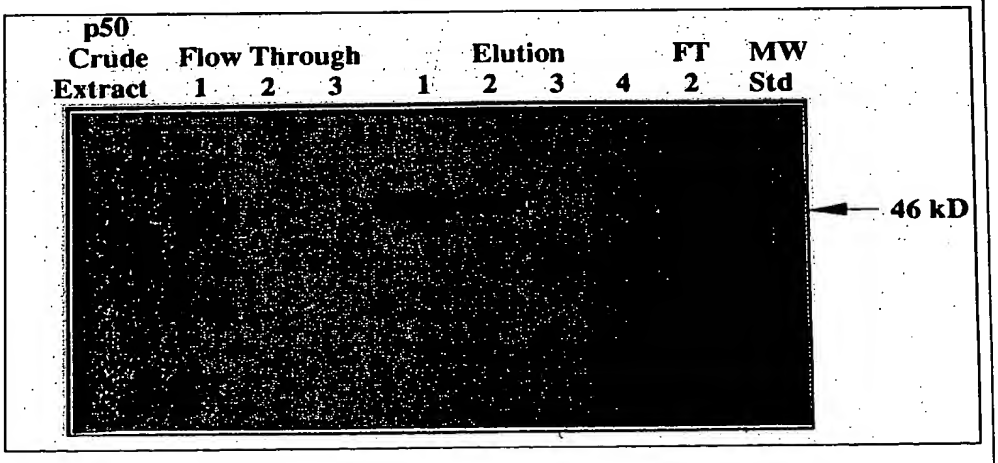
Detection of Anaphylatoxin Receptor with the Anti•FLAG M2 Monoclonal Antibody [4-5, 23]

FIGURE 8:
Gel Retardation



Interaction of Retinoic Acid Receptor with Auxiliary Receptor Protein using the Anti•FLAG M2 Monoclonal Antibody [8-9]

FIGURE 9:
Affinity Purification



Purification of FLAG•BAP Fusion Protein with Anti•FLAG M2 Affinity Gel

FLAG PRODUCTS

A range of products are offered for the expression, immunological detection and affinity purification of FLAG fusion proteins.

FLAG Expression Vectors:

A family of expression vectors is offered for regulated expression of amino-terminal, carboxy-terminal or FLAG•Shift fusion proteins in *E. coli*.

Storage and Stability:

Supplied in:.....1 mM Tris•HCl, 0.1 mM EDTA at pH 8.0
Storage: .....Short term (≤2 weeks) +4°C
Long term (≥2 weeks) -70°C
Stability: .....1 year

An ORF (open reading frame) can be cloned into an amino-terminal (FIGURE 10a) or carboxy-terminal (FIGURE 10b) FLAG Expression Vector if the phase of the reading frame is known and/or the amino-terminus of the FLAG fusion protein must be precisely defined. Expression is possible using the FLAG•Shift (FIGURE 10c) Expression Vectors[17] if the amino-terminus need not or cannot be precisely defined and/or the phase is unknown. FLAG•Shift Expression Vectors contain a "shift" sequence that allows expression of an ORF without regard to the reading frame in which it was initially cloned. The shift sequence consists of a string of 12 thymidines which causes translational frameshifting[31-36] and results in the synthesis of FLAG fusion proteins in all three reading frames. The result is a FLAG fusion protein containing the amino acid sequence of the correctly phased ORF.

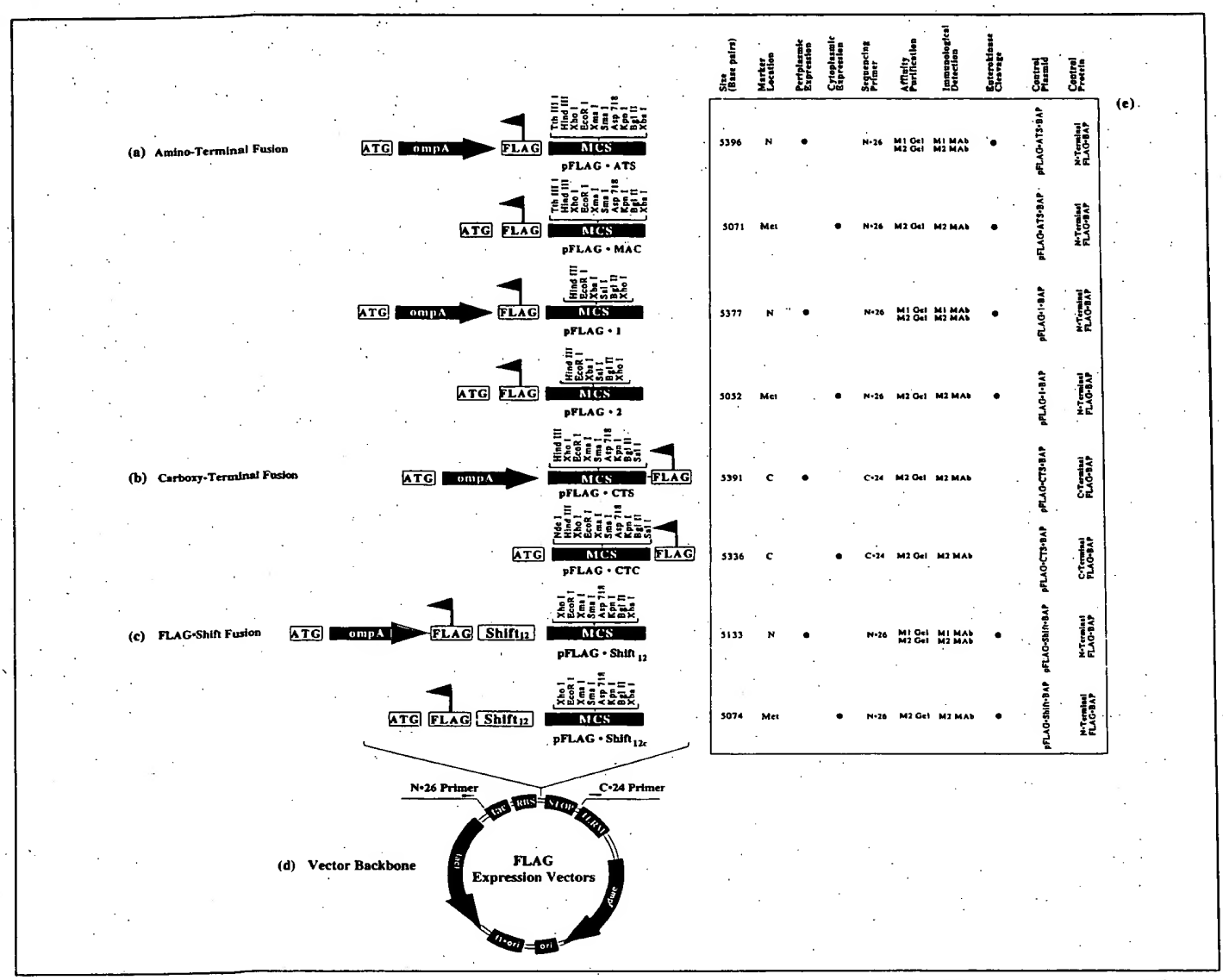
ORDERING INFORMATION

Product	Catalog #	Quantity	Price
For Amino-Terminal Fusion:			
pFLAG•1 Expression Vector *(Not available in kit)*			
pFLAG•2 Expression Vector *(Not available in kit)*			
pFLAG•MAC Expression Vector			
pFLAG•MAT Expression Vector			
For Carboxy-Terminal Fusion:			
pFLAG•CTC Expression Vector	IB13060	10 ug	115.00
pFLAG•CTS Expression Vector	IB13061	10 ug	115.00
For Frameshift Expression:			
pFLAG•Shift-12 Expression Vector			
pFLAG•Shift-123 Expression Vector			

Each of the amino-terminal, carboxy-terminal or FLAG•Shift Expression Vectors are offered as pairs. Selection of one member of the vector pair depends on whether cytoplasmic or periplasmic expression of a FLAG fusion protein is desired. One member of each pair encodes the OmpA signal peptide for periplasmic expression of a FLAG fusion protein. The second member lacks the OmpA coding sequence and results in cytoplasmic expression of a FLAG fusion protein. All vectors share similar multiple cloning sites (MCS) which allow transfer of an ORF between each member of the FLAG Expression Vector family.

This feature allows transfer of an ORF with little or no manipulation prior to cloning. Expression vectors offered in kits contain an MCS with restriction sites represented in each of the three reading frames. Restriction enzyme digestion of a site within each MCS can yield 5′, 3′ or blunt ends. The pFLAG•1 and pFLAG•2 Expression Vectors, which are only available separately, share an identical MCS, but all sites are in the same phase. Restriction enzyme digestion of any site within the MCS of these vectors yields a four base, 5′ overhang.

FIGURE 10:
FLAG Components for Immuno-Affinity Purification and Detection of FLAG Fusion Proteins



IBI E. coli FLAG Expression Vectors differ in the expanded regions shown above: (10a): Amino-terminal FLAG fusion proteins are expressed in the periplasm by cloning and phasing an open reading frame (ORF) into pFLAG•ATS or pFLAG•1. pFLAG•MAC or pFLAG•2 are used for cytoplasmic expression. (10b): Carboxy-terminal FLAG fusion proteins are expressed into the periplasm by cloning an ORF into pFLAG•CTS. An ORF is cloned into pFLAG•CTC for expression within the cytoplasm. (10c): An ORF of unknown reading frame can be expressed in the periplasm by cloning into pFLAG•Shift$_{12}$. The ORF is cloned into pFLAG•Shift$_{12c}$, for expression within the cytoplasm. IBI E. coli Expression Vectors share a common backbone (10d) which includes: (i) tac promoter for controlled, high level expression of FLAG fusion proteins by de-repression with IPTG; (ii) Shine-Dalgarno ribosome binding site; (iii) translational stop codons; (iv) rrnB tandem transcriptional terminators; (v) ampicillin resistance gene for plasmid selection; (vi) colE1 double stranded origin of replication; (vii) f1•ori single stranded origin of replication via super-infection with the M13KO7 Helper Phage and (ix) lacI repressor gene for repression of the lac promoter. De-repression of lacI is with IPTG. (10e): Table lists FLAG system components that are recommended for handling of FLAG fusion proteins expressed by each of the IBI E. coli FLAG Expression Vectors. Abbreviations and symbols: •, yes; Met, Met•amino-terminal FLAG fusion protein; N, amino-terminal FLAG fusion protein; C, carboxy-terminal FLAG fusion protein; MAb, monoclonal antibody.

FLAG PRODUCTS

Sequencing Primers:

The FLAG sequencing primers are useful for DNA sequence determination of FLAG fusion junctions of coding sequences cloned into IBI *E. coli* FLAG Expression Vectors.

Storage and Stability:

Supplied in: .....10 mM Tris•HCl, 1.0 mM EDTA at pH 8.0
Storage: .....-20°C
Stability: .....1 year

ORDERING INFORMATION

Product	Catalog #	Quantity	Price
N•26 Sequencing Primer	IB13013	1 ug	95.00
C•24 Sequencing Primer	IB13012	1 ug	95.00

The N•26 primer is designed for DNA sequencing of amino-terminal FLAG fusion junctions.

The C•24 primer is designed for DNA sequencing of carboxy-terminal FLAG fusion junctions.

Both primers are sufficiently thermostable for PCR* cloning.

** The GeneAmp PCR process is covered by U.S. Patents owned by Hoffman-LaRoche Inc. and Hoffmann-LaRoche Ltd.*

Anti•FLAG Monoclonal Antibodies:

The Anti•FLAG Monoclonal Antibodies are useful for the immunological detection of FLAG fusion proteins in a variety of applications.

Anti•FLAG M1 Monoclonal Antibody:

The Anti•FLAG M1 (IgG_{2b}) Monoclonal Antibody binds the FLAG peptide only when it is located at the free amino-terminus of a FLAG fusion protein. (FIGURE 11). The Anti•FLAG M1 Monoclonal Antibody binds the FLAG peptide in the presence of calcium, but disassociates in its absence[2]. This property is convenient for the affinity purification of FLAG fusion proteins.

Anti•FLAG M2 Monoclonal Antibody:

The Anti•FLAG M2 Monoclonal Antibody can bind an available FLAG peptide whether it is located at the free amino terminus, Met•amino-terminus, internal site or carboxy-terminus of a FLAG fusion protein. (FIGURE 11). The Anti•FLAG M2 (IgG_1) Monoclonal Antibody does not bind to the FLAG peptide in a calcium dependent manner. It is disassociated from the FLAG fusion protein by competitive binding with FLAG peptide[3]. Amino-terminal, Met-amino-terminal and carboxy-terminal FLAG fusion proteins can be purified using affinity chromatography by elution with FLAG peptide.

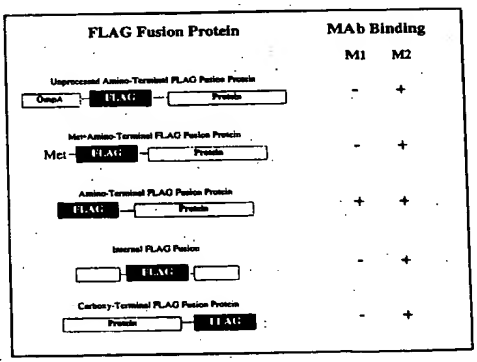
ORDERING INFORMATION

Product	Catalog #	Quantity	Price
Anti•FLAG M1 Monoclonal Antibody	IB13001	200 ug	125.00
	IB13005	1 mg	195.00
	IB13007	5 mg	295.00
Anti•FLAG M2 Monoclonal Antibody	IB13010	200 ug	125.00
	IB13025	1 mg	195.00
	IB13026	5 mg	295.00

Storage and Stability:

Supplied in: .....PBS/A (137 mM Sodium Chloride, mM Potassium Chloride, 10 mM Phosphate buffer salts at pH 7.4 in 0.2 % azide)
Storage: .....Short Term (< 1 week) +4°C
Long Term (> 1 week) -20°C
Stability: .....1 year

FIGURE 11:
Binding of Anti•FLAG M1 and M2 Monoclonal Antibodies to the FLAG Peptide



The Anti•FLAG M1 Monoclonal Antibody can bind to the eight amino acid FLAG peptide only when it is located at the free amino terminus of a FLAG fusion protein. The Anti•FLAG M2 Monoclonal Antibody can bind an available FLAG peptide at any location.

FLAG PRODUCTS

Anti•FLAG Affinity Gels:

The Anti•FLAG Affinity Gels are useful for the affinity purification of FLAG fusion proteins. Each is supplied as a suspension of agarose beads covalently linked to the Anti•FLAG M1 or M2 Monoclonal Antibody. Their use involves flowing the FLAG fusion protein preparation through the packed gel in a column, washing away contaminating proteins and eluting the purified FLAG fusion protein with an appropriate reagent.

Anti•FLAG M1 Affinity Gel:

Appropriate reagents to elute a FLAG fusion protein from the Anti•FLAG M1 Affinity Gel include: **(1)** EDTA which chelates calcium or **(2)** Competitive elution with FLAG peptide.

Anti•FLAG M2 Affinity Gel:

FLAG fusion proteins are eluted from the Anti•FLAG M2 Affinity Gel via: **(1)** Competitive elution with FLAG peptide or **(2)** Acid elution with glycine at pH 3.0. EDTA elution is not useful since the Anti•FLAG M2 Monoclonal Antibody does not bind to the FLAG marker in a calcium dependent fashion.

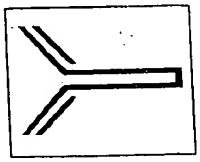
ORDERING INFORMATION

Product	Catalog #	Quantity	Price
Anti•FLAG M1 Affinity Gel	IB13002	1 mL	225.00
	IB13008	5 mL	475.00
	IB13009	10 mL	950.00
	IB13013	25 mL	1350.00
Anti•FLAG M2 Affinity Gel	IB13020	1 mL	225.00
	IB13021	5 mL	475.00
	IB13022	10 mL	950.00
	IB13023	25 mL	1350.00

Storage and Stability:

Supplied in:.....PBS/A (137 mM Sodium Chloride, 3 mM Potassium Chloride, 10 mM Phosphate buffer salts at pH 7.4 in 0.2 % azide)
Storage:.....+4°C. Do not freeze.
Stability:.....1 year

FLAG PRODUCTS



FLAG Peptide:

The FLAG octapeptide is a convenient and gentle reagent to elute a FLAG fusion protein from both the Anti•FLAG M1 and M2 Affinity Gels. Proteins may be efficiently eluted with a 5-25 fold mole excess of the peptide.

ORDERING INFORMATION

Product	Catalog #	Quantity	Price
FLAG Peptide	IB13070	4 mg	95.00

Storage and Stability:

Supplied as: .....Lyophillized powder
Storage: .....+4°C. Dessicated.
Stability: .....1 year

Enterokinase:

A purified preparation of enterokinase is provided for the removal of the FLAG peptide from N-terminal and Met•amino-terminal FLAG fusion proteins.

ORDERING INFORMATION

Product	Catalog #	Quantity	Price
Enterokinase	IB13005	1 U	95.00

Storage and Stability:

Supplied as: .....Lyophillized powder
Storage: .....-20°C.
Stability: .....1 year

FLAG•BAP Control Proteins:

The 55 kD, FLAG•BAP Control Proteins can be used to assure the functional integrity of the Anti•FLAG M1 or M2 Affinity Gels, the Anti•FLAG M1 and M2 Monoclonal Antibodies and enterokinase. They are also useful controls on electrophoresis gels, Western, dot or slot blots.

ORDERING INFORMATION

Product	Catalog #	Quantity	Price
Amino-Terminal FLAG•BAP Control Protein	IB13200	100 ug	95.00
Carboxy-Terminal FLAG•BAP Control Protein	IB13201	100 ug	95.00

Storage and Stability:

Supplied in: .....10 mM Tris•HCl,
120 mN NaCl,
0.05 mM ZnCl,
50% Glycerol at pH 8.0
Storage:.....Short term (≤3 weeks) -20°C
Long term (≥3 weeks) -70°C
Stability: .....1 year